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## THE ETIOLOGY OF ACUTE RHEUMATISM, ARTICULAR AND MUSCULAR.\*

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The view that rheumatism is due to a specific streptococcus as claimed by Poynton and Paine<sup>1</sup> and others, has not been generally accepted because many investigators (Phillip,<sup>2</sup> Cole,<sup>3</sup> Beattie,<sup>4</sup> and others) failed entirely to obtain streptococci from the joints while others as Loeb<sup>5</sup> only rarely succeeded. No one has isolated the organism in a considerable number of consecutive, uncomplicated cases of rheumatism during life.

I now wish to report the results of cultures from the blood and the lesions in cases of articular and of muscular rheumatism, the chief characteristics of the organisms isolated, and results of experiments.<sup>6</sup>

### TECHNIC OF CULTURES.

The skin was sterilized with tincture of iodin, and the joints aspirated with a glass syringe through a needle of small caliber. The pain was slight and the patients were afforded relief from pain whenever the amount of fluid obtained was considerable. The fluid was emptied into sterile test tubes through the flamed end of the syringe and not through the needle. The mouth of the test tubes was thoroughly flamed and kept open as short a time as possible. The material was then taken to the laboratory and cultures made as soon as possible. In a number of cases cultures were made immediately at the bedside, but the number of organisms isolated was no greater than in those made later in the laboratory. At first the cultures were made both under aerobic and anaerobic conditions in plain broth, ascites broth, ascites-dextrose broth, blood broth, litmus milk, ascites fluid (heated and unheated), Löffler's blood serum slants, blood agar plates and slants.

The fact that the lesions in rheumatism occur in relatively avascular regions suggested that the infecting organism might be sensitive to oxygen pressure. In order to meet this possibility the joint fluid was inoculated into tubes containing tall columns of ascites-dextrose agar, the top of which would give aerobic conditions, while the bottom would be anaerobic on account of the oxygen consuming property of the leukocytes

\* Received for publication September 10, 1913.

<sup>1</sup> *Lancet*, 1900, 2, p. 861; 1910, 1, pp. 152 and 1528.

<sup>2</sup> *Deutsch. Arch. f. klin. Med.*, 1903, 76, p. 150.

<sup>4</sup> *Brit. Med. Jour.*, 1906, 2, p. 1781.

<sup>3</sup> *Jour. Infect. Dis.*, 1904, 1, p. 714.

<sup>5</sup> *Arch. Int. Med.*, 1908, 2, p. 266.

<sup>6</sup> A preliminary report was published in *Jour. Am. Med. Assn.*, 1913, 60, p. 1229.

in the exudate, the space between representing a gradual transition from one condition to the other. It was soon found that this method gave by far the best results. The standard 2 per cent agar, 0.4 to 0.6 per cent acid to phenolphthalein, and containing 0.2 to 1 per cent dextrose, was used, 7 to 8 c.c. being placed in each tube. The agar was melted and boiled for a time to drive off oxygen, cooled to 50° C., and 2 to 3 c.c. heated ascites fluid (60° C. 24 hours) added to each. After being cooled to 40° C., from 0.1 to 1 c.c. of the joint fluid was inoculated, depending on the amount at hand, and mixed with the agar. The tube was plunged into cold water to "set" and then placed at 37° C. Growth usually was present at the end of 48 hours, but sometimes it would not take place until after three or more days. The cultures (aerobic and anaerobic) made in liquid and on the surface of solid media yielded positive results in only an occasional case, while the method just described yielded positive results in the case of one or more joints in 14 of 16 cases. That the oxygen requirement is the chief factor to explain this difference in my results and the negative results of others is indicated also by the fact that the colonies never developed above 0.5 cm. from the top and never below 2 cm. from the bottom of the agar tubes. The largest number of colonies developed between 1.5 cm. from the top and 3.5 cm. from the bottom.

#### THE RESULTS.

An account of the cultures of the joint fluid from one case will illustrate the results. There were aspirated 18 c.c. of fluid which was inoculated into plain broth, dextrose broth (with and without ascites fluid), on the surface of blood agar slants (aerobic and anaerobic), and into ascites-dextrose agar, four tubes of which received one-third of the fluid. All liquid cultures remained sterile, one blood agar slant (anaerobic) yielded a positive result, while in the ascites-dextrose agar on the other hand there developed an average of nine colonies per cubic centimeter of fluid. No colonies developed above one centimeter from the top of the agar or below three centimeters from the bottom.

The joint fluids obtained were only moderately turbid, due to the presence of leukocytes and a few endothelial cells. Smears showed unmistakable diplococci in only three cases. The two cases in which cultures gave negative results were fever free and convalescing at the time the cultures were made. All the cases were typical examples of acute rheumatic fever and were not selected except as to time.<sup>1</sup>

It was early learned that cultures made soon after a joint

<sup>1</sup> The cases studied occurred chiefly in the Cook County and Presbyterian Hospitals, and I wish here to express my appreciation to the medical staffs for the privileges accorded me, and to Dr. Falls and Dr. Phemister for aid in obtaining material for cultures.

became involved or soon after an exacerbation showed more organisms than at other times. The number of organisms obtained from the fluid, however, was never more than 15 per cubic centimeter.

The results in the following case are interesting. Carpenter, 36 years old, began to have severe pain in groins and right hip after severe exposure four days before. Left knee became very painful and swollen the following day, and the right knee two days later. The left wrist became involved the following night. Free fluid was easily demonstrable in the joints involved and a red, very tender, swollen area was found over the lower end of the ulna. Both knees and wrist joints were aspirated. A needle was inserted in various directions into the red area over the ulna and by means of strong suction a small quantity of bloody fluid was obtained. This was inoculated in ascites-dextrose agar at once. Cultures from the joint fluid were made in the same way. The fluid from the right knee yielded two colonies per 20 c.c., from the left knee two colonies per 12 c.c., from the wrist three colonies per 2 c.c., while the small amount (approximately 0.05 c.c.) of bloody fluid from the red area yielded 10 colonies.

In the cultures from a number of joints there developed a few colonies of a large gram-positive aerobic bacillus which resembled *B. subtilis*. This was probably due to air contamination.

Blood cultures were made in seven cases. In all fever was present, ranging from 99.5° to 102° F. when the cultures were made. In only one was endocarditis present. Three of the cultures were made in the usual way, the blood being inoculated at once into plain broth, ascites-dextrose broth and into plain and ascites-dextrose agar. Two of these cultures were sterile while the third yielded two very small colonies in the fibrin clot in the dextrose broth cultures. In the other four cases the cultures were made by drawing 30 c.c. of blood into 15 c.c. of 2 per cent sodium citrate in NaCl solution. In order to get rid of the hemoglobin the red blood corpuscles were now hemolyzed by adding approximately 100 c.c. of distilled water. This fluid was now centrifugated at high speed and the sediment inoculated at once in broth and ascites-dextrose agar. From two to 12 colonies developed by this method in three of the cases. The cultures in the broth proved sterile in all four. Simultaneous blood and joint cultures were made in four cases. The joint cultures were positive in all of these but blood cultures were sterile in two. The organism was isolated from the tonsil at the height of the attack in one case.

While the tonsils probably are the common portal of entry of the organisms in rheumatism, and I may venture to say probably

a place where the organisms acquire the peculiar properties necessary to produce the disease known as rheumatism, other foci may be responsible, as shown by the following case:

A middle-aged man had a typical recurring attack of rheumatism one year after complete extirpation of the tonsils. (Other foci of infection, such as the prostate, were excluded.) The attack followed an indefinite intestinal disturbance. An organism resembling in every way, those obtained from the joints in the cases in which muscle

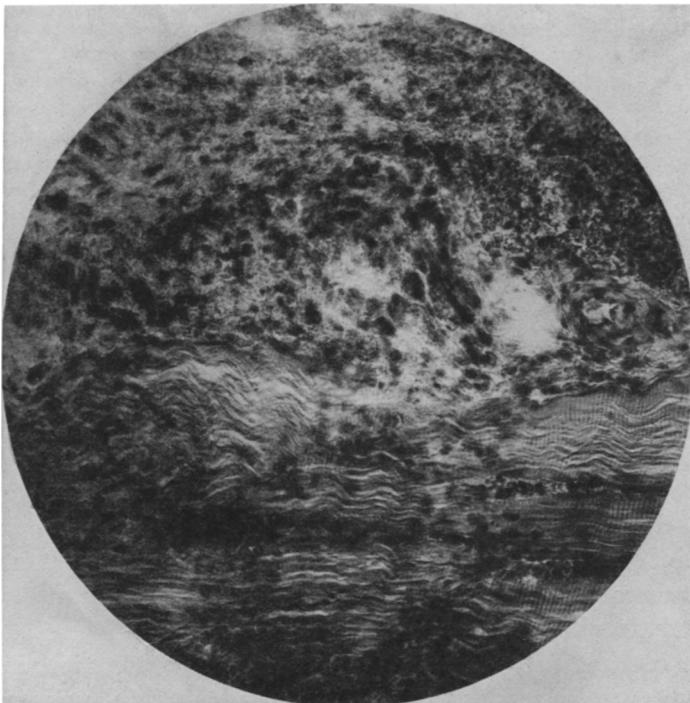


FIG. 1.—Microphotograph of human (biceps) muscle showing marked degeneration of muscle fibers leukocytic infiltration, and hemorrhage. Frozen section. Hematoxylin and eosin.  $\times 230$ .

involvement was present was isolated from the flakes of mucus in the stools on three occasions. The patient was injected with a vaccine of the organism from other cases and with one prepared from the culture obtained from the feces. Recovery was rapid. After the joint symptoms had disappeared examination of the stool failed to show the coccus. The strain when isolated produced arthritis, pericarditis and endocarditis, while after one animal passage it acquired an affinity for the muscles, producing now myositis, myocarditis, endocarditis, and arthritis. Cultures from the blood during an attack of rheumatism in another case showed a similar organism.

The results of the cultures in the following case of muscular rheumatism are of special interest. Negro, laborer, 35 years old, admitted to the Cook County Hospital,

May 13, 1913, service of Dr. Patten. He had been ill for 12 days; illness began suddenly, after exposure to wet and cold, with severe pain and swelling first of left elbow and wrist joint. In a few days the knees became involved. When admitted the knees were still swollen and tender but soon improved. On May 17, the following notation was made: the day after admission the patient developed severe pain on the inner and upper aspect of right thigh. On May 15, there developed severe pain in the muscles of the neck and in the occipito-frontalis, associated with tenderness on pressure, and severe general muscular pain. On May 16, there developed sudden pain in lower third of the biceps of right arm and upper third of right forearm, with fever. Over these areas a distinct swelling was present.

The areas were cocaineized and pieces of muscle removed. The muscles were hemorrhagic and edematous. The hemorrhagic areas were in close proximity to the tendinous parts but widely separated from the joints. Microscopic sections (see Fig. 1) revealed marked degeneration of muscle, extravasation of blood, and leukocytic infiltration. The patient was given large doses of a vaccine prepared from various strains from rheumatism and recovered without suppuration in the muscles and without developing further lesions.

Cultures were made by placing small pieces and emulsions of the muscle on the surface of blood agar slants and plates, dextrose-agar slants, Löffler's blood serum slants, into ascites-dextrose broth and into ascites-dextrose agar. The only growth on the surface of the various solid media and in the broth were three colonies of staphylococci, probably a contamination. The ascites-dextrose agar inoculated with material from the biceps gave 15 colonies, while from the forearm five colonies were obtained. These were all within a space 0.5 cm. from the free surface and 3 cm. from the bottom of the tube. The culture from the blood was made by getting rid of the hemoglobin and planting the sediment into ascites-dextrose agar. Fifteen colonies developed. This organism corresponds very closely indeed with those obtained from the joints in cases in which muscle involvement is present.

In two other cases of articular rheumatism in which distinct muscular involvement was present attempts were made to isolate the organism from excised pieces of muscle but without result, and sections did not show any lesions. The areolar tissue overlying a piece of the left suprascapular muscle in one case, however, showed a circumscribed area of leukocytic infiltration.

#### CHARACTERISTICS OF THE STREPTOCOCCI.

Three types of cocci have been obtained from the joints in rheumatism.

The strains from five cases, in none of which were muscles involved, produced green on blood agar, formed long chains of diplococci and large clumps in broth; when injected into animals they produced a nondestructive arthritis, pericarditis and endocarditis. The diplococci were distinctly larger and more uniform in size than those of the *Str. viridans*.

In six cases, all of which showed more or less muscular involvement in conjunction with arthritis, the organisms isolated produced

a slight but hazy hemolysis on blood agar plates, formed short chains and diplococci in broth, and when injected intravenously in rabbits commonly produced arthritis, endocarditis, some pericarditis, a myositis, and often a most pronounced myocarditis. They are the same size as hemolytic streptococci but the diplococcus arrangement in the chains is more marked.

In three cases, none of which showed symptoms referable to the muscles, the organisms produced small, grayish colonies on blood agar plates without perceptibly affecting the medium. They formed clumps of small micrococci and occasionally diplococci and short chains. One of these strains produced arthritis, endocarditis, and pericarditis but no muscle lesions.

These results would seem to explain why different observers have named organisms like these "*Streptococcus*," "*Diplococcus*," or "*Micrococcus rheumaticus*" depending on the particular type with which they happened to be working. The virulence of all three types is of a low order. The third group seems to be the least virulent, the first group occupying a middle position, those from cases of muscular and articular rheumatism being the most virulent. Tests of their susceptibility to phagocytosis place them in the same order. They are not bile soluble and they autolyse slowly in NaCl solution. There is no capsule.

A number of strains were lost in the first or second cultures, but they may live for a long time on blood agar and other media.

When first isolated they are all characterized by a marked capacity to multiply at a low temperature. They are very sensitive to oxygen pressure. Three of the strains of the first group and all of the strains which produced muscle lesions ferment mannite but not inulin and produce a high acidity in dextrose broth (5-7 per cent). This is particularly true of the strains which produce muscle lesions. They produce marked clouding on ascites-dextrose agar. At first they grow readily in filtrates of streptococcus broth cultures, whereas later they do not.

When injected intravenously in animals they produce lesions which correspond quite closely to those found in man.

After cultivation from one to eight months the capacity to grow at a low temperature, the sensitiveness to oxygen, the excessive

production of acid in dextrose broth, and the simultaneous affinity for joints, pericardium, endocardium, and myocardium are found to have largely or entirely disappeared.

For the details of experiments on mutation of these cocci reference is made to the paper on "Transmutations within the Streptococcus-Pneumococcus Group" (See p. 1).

Here the following statement may be made: By appropriate means strains of the three varieties have been converted each one into the other. Thus Strain 734 from articular rheumatism, produced green colonies on blood agar, long chains in broth and arthritis, endocarditis, and pericarditis in rabbits (see experiments, Rabbits 418, 374, 382, 356, etc.). After "soaking" the strain in distilled water for three weeks it produced a narrow zone of hemolysis on blood agar, diplococci and short chains in broth, and in addition to arthritis, endocarditis, and pericarditis produced also marked myositis and myocarditis just as the strains as isolated from cases of muscular and articular rheumatism (see experiments, Rabbit 428). After prolonged cultivation the strains which produced green at first come to resemble *Str. viridans* in morphology, in cultural, and pathogenic properties. The strains which produced a slight, hazy hemolysis at first now usually produced a wide zone of hemolysis, and when injected produced arthritis but no endocarditis and pericarditis. By means of animal passage strains of each group have been converted into typical pneumococci. This is most readily accomplished with the strains which produce the diplococci and short chains.

#### RECORDS OF EXPERIMENTS WITH STRAINS AS ISOLATED FROM ARTICULAR RHEUMATISM.

##### Rabbit 418:

March 5, 1913. Injected intravenously with the growth from 75 c.c. of ascites-dextrose broth of Strain 734. Death in 10 minutes. Large subendothelial hemorrhage in septum and papillary muscles of left ventricle. A few small hemorrhages in right auricle and on the cut surface of the myocardium. Circumscribed hemorrhages in the parietal pericardium. Fluids in the joints clear but cultures from one joint gave green-producing colonies of diplococci.

##### Rabbit 374:

February 11. Injected intravenously with the growth of 30 c.c. ascites-dextrose broth of Strain 734.

February 12. Dead. Two hemorrhages at base of pulmonary semilunar cusps and one near the free margin of adjoining cusps. Moderate amount of turbid fluid

in pericardium and joints. A number of hemorrhages in the periarticular structures, and in one joint a small hemorrhage in the intracapsular ligament. A few colonies of streptococci obtained from the blood, the pericardial fluid, and one joint.

*Rabbit 382:*

February 13. Injected in ear vein with the growth of 35 c.c. ascites-dextrose broth of Strain 734<sup>2</sup>.\* Died the next day. Multiple, embolic hemorrhage in mitral and tricuspid valves; small hemorrhages in myocardium and glomerular tufts. Mucous membrane of duodenum and of appendix hemorrhagic. Fluid from both knees contained a moderate number of leukocytes. A few colonies of streptococci obtained from blood, and from one joint.

*Rabbit 356:*

January 29. Injected intravenously with the growth from 25 c.c. ascites-dextrose broth of Strain 734 as isolated from the joint.

January 31. Lame, left front ankle joint swollen.

February 3. Dead. Pericardium distended with moderately turbid fluid with fibrin and leukocytes; fluids from left front ankle and other joints turbid. Smears from ankle joint showed a few diplococci. Two small subendothelial vegetations on the tricuspid valve. Cultures gave one green, nonadherent colony from blood, about 50 from pericardial fluid, and 150 from left ankle joint; no colonies from other three joints. The colonies from the joint fluid were quite different from those from blood and pericardial sac, being more elevated, less moist, surrounded by a narrow zone of hemolysis, and smears showed larger clumps of streptococci.

*Rabbit 376:*

February 11. Injected intravenously with the growth from 30 c.c. of ascites-dextrose broth of Strain 734<sup>3</sup>.

February 14. Lame, right knee joint swollen.

February 17. Dead. No pericarditis, no visible myocarditis, small vegetation at apex of papillary muscle in left ventricle. Fluid from three joints turbid, from two other joints clear. Smears from the right knee joint showed large number of leukocytes, no reds, a few diplococci, mostly within cells. Cultures from blood, pericardial fluid, pelvis of kidney, and two joints sterile. Left knee joint gave a moderate number of green colonies.

*Rabbit 378:*

February 11. Injected intravenously with 5 c.c. of an emulsion in NaCl solution of a kidney from a guinea-pig which died immediately after an injection of Strain 734. The kidney was removed in a sterile manner and incubated for 24 hours in NaCl solution. A marked growth took place.

February 14. Marked swelling of left knee joint. Animal chloroformed. There were edema and numerous hemorrhages just where the muscles merge into the tendon sheaths and periosteum about the left knee. Smears from these areas showed many organisms. The joint fluid was turbid, containing many leukocytes, but smears showed only a small number of organisms. On opening the joint, hemorrhages were found in the intracapsular ligament and along the line of attachment of the ligaments. The opposite knee showed a similar condition except that the hemorrhages were less

\* The figure to the right and above the number of the strain, indicates the number of animal passages.

marked, the joint fluid less in amount and less turbid. Three other joints were punctured but only one gave a turbid fluid. Cultures from the blood and fluid from three joints sterile. Large number of colonies from edematous and hemorrhagic areas, moderate number from fluid in left knee and few from right knee.

*Rabbit 417:*

March 5. Injected intravenously with the growth from 40 c.c. of ascites-dextrose broth of Strain 734<sup>8</sup>.

March 7. Injection repeated.

March 8. Dead. Hemorrhages about joints; most marked about knee joints; intracapsular ligament of right knee showed two small hemorrhages; joint fluids turbid; small round subendothelial, grayish white, nodules in tricuspid valve; pericardial sac contained a moderate amount of slightly turbid fluid; small punctate hemorrhages in cortex of kidney; small punctate scleral hemorrhages in the limbus; small nodules in iris. Smears from joint fluid showed a few, while those from the hemorrhagic areas showed a larger number of diplococci. Cultures from blood and knee joints gave a few colonies while the bloody fluid around joints and the nodules in iris gave large number of green-producing colonies of gram-staining diplococci and chains.

RECORDS OF EXPERIMENTS WITH STRAINS FROM CASES OF ARTICULAR AND MUSCULAR RHEUMATISM AND WITH OTHER STRAINS WHICH HAVE BEEN MADE TO RESEMBLE THESE.

*Rabbit 418:*

March 10. Injected intravenously with growth from 45 c.c. ascites-dextrose broth of Strain 734 after it was made to resemble the strains from muscular rheumatism.

March 12. Dead. Numerous, elongated grayish white areas, 2-10 by 1-2 mm., running parallel with the muscle fibers in the skeletal muscles, especially in the intercostal muscles, the flat muscles of the abdomen (see Fig. 2), neck and shoulder, in the muscles of the back and in the more tendinous portions of the muscles of the extremities and the diaphragm. A small number were found in the upper end of the esophagus, the muscles about the larynx, but only one in each of the masseters. Smears from these areas in the muscles showed a moderate number of gram-staining diplococci, and leukocytes. The character of these lesions is illustrated in Figure 3. Such lesions were not found in the muscular coat of the stomach, or intestines, but several small grayish white nodules were found in the wall of the bladder. Small numbers of grayish white but less circumscribed and more irregular areas were found in the myocardium of the right ventricle, but not in the left ventricle, and there were small subendothelial grayish white nodules in the tricuspid valve and chordae tendineae. No hemorrhages about joints but the fluid in three of five joints was turbid. The mucous membrane of the appendix showed marked hyperemia with a number of small ulcers. The kidney showed whitish, linear areas in the medullary portion; cortex normal. At the apex of one of the areas there was a small ulcer in the pelvis of kidney, the smears showing diplococci, short chains and leukocytes. The mucous membrane of the pelvis of the kidneys appeared edematous. The urine contained a large number of leukocytes, columnar epithelial cells, and some diplococci and short chains. Cultures on blood agar plates gave a small number of slightly hemolysing colonies from the blood, one joint, and piece of crushed muscle not containing visible lesions, while cultures from the other turbid joint fluid, the crushed vegetation, pelvis of kidney, whitish area in

medullary portion of kidney, the urine, and piece of muscle containing one of the grayish streaks showed a large number of similar colonies.

*Dog 25:*

March 26. Injected into vein of hind leg with the growth of 240 c.c. of ascites-dextrose broth of Strain R<sub>51A<sup>20</sup></sub>. This strain, originally a pneumococcus, was made to resemble the strains from muscular rheumatism.



FIG. 2.—Photograph of lesions in abdominal wall in Rabbit 418 (see protocol).  $\times 5.6$ .

March 27. Lame in left front leg, weakness in extremities.

March 28. Turbid fluid from both knee joints. Cultures gave a few very small slightly hemolysing colonies.

March 31. Right front wrist swollen, fluid turbid. Very tender over muscles of back and neck. Pressure here and over the tendinous portion of muscles of extre-

ties caused animal to yelp, whereas same amount of pressure over belly or muscles of extremities did not. Marked tenderness in groin. Animal chloroformed and muscles exposed. A number of grayish white areas similar to those in rabbits were found and two were excised. Cultures gave streptococci and sections showed inflammatory areas.

April 3. No lameness but animal seemed weak; walked about very little, ate little, and drank water only.

April 8. Found dead, body still warm. Tissues everywhere pale. A large number of grayish white streaks in muscles about neck, shoulder, under shoulder

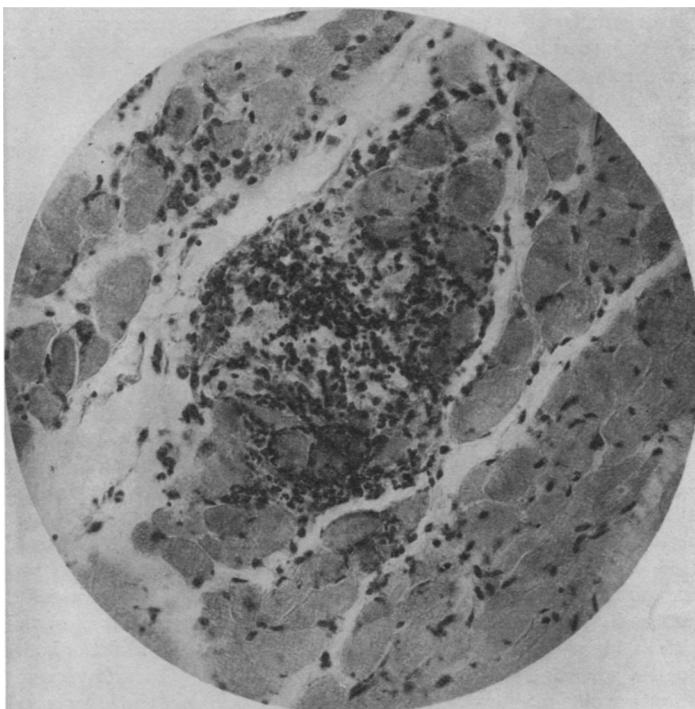


FIG. 3.—Microphotograph of lesion in muscle (trapezius) of Rabbit 418. Death 48 hours after injection. Note degeneration of muscle fibers, leukocytic, and round cell infiltration. Hematoxylin and eosin.  $\times 230$ .

blade, intercostal muscles, diaphragm, and the more tendinous portion of the muscles of the extremities. One distinct abscess about shoulder joint. Joint fluids clear. Myocardium grayish red and flabby. A number of whitish areas were found in the wall of the right ventricle and in the apex of the left papillary muscle. Pericardium and endocardium normal. Kidneys showed yellowish white linear areas radiating from pelvis where there were a number of small ulcers. Pelvic mucous membrane was edematous. Urine contained moderate number of leukocytes and columnar epithelium. Large, deep ulcer of duodenum ( $1 \times 0.5$  cm.) two centimeters beyond

pyloric ring. The margins were undermined and infiltrated. Local peritonitis with adhesions to omentum; another, similar ulcer three centimeters from pyloric ring. Intestines contained large amount of partially digested dark red blood. Thyroid on cross section showed several whitish areas similar to those in the muscles. Liver showed a moderate fatty degeneration. The gall bladder was filled with bile containing mucus and flakes of pus. The lungs, adrenals, appendix, meninges and brain showed no gross changes. Cultures on blood agar plates from blood, joints, and pericardial fluids were sterile; from pelvis of kidney, white streak in medullary portion, urine and pus from about shoulder joint gave moderate number, while the bile showed a very large number of slightly hemolysing streptococci in pure growth. Sections from the ulcer in the duodenum, stained by Gram-Weigert's method showed a moderate number of deeply staining diplococci in the wall of the ulcer.

*Monkey:*

March 18. Leukocytes, 10,500. Injected with the growth from 100 c.c. of ascites-dextrose broth culture of Strain 734H into vein of forearm.

March 20. Leukocytes, 24,500. Seemed quite well. Both knee joints aspirated and cultures made. Injection repeated.

March 22. Cultures from one joint sterile, those from the other showed eight green-producing colonies. Leukocytes, 9,400. Seemed weak. Anesthetized and a small portion of trapezius excised.

March 24. Suppurative conjunctivitis in left eye. Cultures gave a few green-producing colonies as was also the case with cultures from knee joint.

March 25. Leukocytes, 24,800.

March 31. Seemed very sick; severe diarrhea; took no food.

April 2. Dead. Moderate number of grayish white lesions in intercostal muscles anteriorly, in flat muscles of the groin, in scapular and spinal muscles, in intervertebral and tendinous portion of muscles of extremities. These areas were quite similar to those found in rabbits and dogs. Smears from the areas showed leukocytes but no bacteria. Pericardium contained a small amount of turbid fluid. In the epicardium there were a number of linear scars which joined the right auricle and right ventricle, and inclosed a gelatinous, grayish yellow material, smears of which showed leukocytes and an occasional gram-staining diplococcus. A number of subendothelial, whitish nodules in apices of papillary muscles and posterior leaflet of both mitral and tricuspid valves. Myocardium grayish red and soft. Stomach normal except for the presence of three deep ulcers, two near the pyloric end, the other and largest being in the pyloric ring. All had a clean base, were undermined, and had infiltrated margins. The largest had bridges of mucous membrane over undermined portions. Omentum was adherent to stomach and in the largest ulcer there was a small perforation of the serous coat. Small intestines normal. The mucous membrane of the large intestine was hyperemic. The lymph follicles were swollen and a number showed superficial ulceration; a number of these ulcers appeared directly opposite whitish areas in the serous coat. The lymph glands in the mesentery and about the pyloric end of stomach were enlarged and hyperemic. Joint fluids clear. No lesions of brain and cord. The sheath of sciatic nerve contained a large number of very small, whitish areas. Cultures on blood agar plate showed blood and joints to be sterile; the muscle areas and gelatinous material from epicardium yielded a few colonies of streptococci; lymph glands showed mostly colon bacilli, but a few slightly hemolysing colonies; the material in one ulcer in stomach showed saprophytes and colon

bacilli only, but from a small, thoroughly washed, and crushed piece of the wall of the ulcer there were obtained, in addition to contaminating organisms, 15 colonies of streptococci. The healthy mucous membrane showed no streptococci but contaminating colon bacilli only.

#### GENERAL SUMMARY OF THE ANIMAL EXPERIMENTS.

The muscle lesions have been produced in numerous rabbits, dogs, and one monkey. The character of the lesions was similar in all. The number of lesions varied from three to many hundred. In no instance did they lead to suppuration. The lesions have been obtained with four strains as isolated from joints in cases of rheumatism in which muscular involvement was present, with a strain from articular rheumatism after having been modified, and with six strains of "non-virulent" streptococci after they were made to correspond morphologically, culturally (especially on blood agar and ascites-dextrose agar), in resistance to phagocytosis and virulence, to the strains from cases of muscular and articular rheumatism in man. The localization in the muscles of the "non-virulent" strains of streptococci occurred only after from 12 to 21 animal passages. When the various strains produced myositis their affinity for the muscles was so marked that each of a series of animals when injected with the same strain, not only developed the lesions, but the number was quite in proportion to the size of the dose injected. After one or two animal passages this affinity disappeared and it was impossible again to produce the muscle lesions. Owing to the fact that the grade of virulence of the organisms is such that they tend to disappear from the general circulation it was possible to study the exact relation of the organism to the lesions produced. As illustrated by the experiments, streptococci have been found with leukocytes in smears from the lesions and the former have been shown to be alive by cultures, smears and cultures from adjacent muscle either being entirely negative or showing a much smaller number of bacteria. Gram-staining diplococci have been found in sections. The organisms in the muscle lesions were most numerous 48 hours after injection. Small linear scars were formed eventually.

A study of the sections of muscles with lesions shows that there is produced first a small hemorrhage. The muscle fibers then

lose their striations, stain poorly, become granular and break up into fragments as a rather sharp leukocytic infiltration appears (see Fig. 3). The number of organisms is greatest at this time, after which they gradually disappear without causing suppuration. The leukocytes now give way to larger mononuclear cells and as connective tissue is being formed there is found a deeply basic

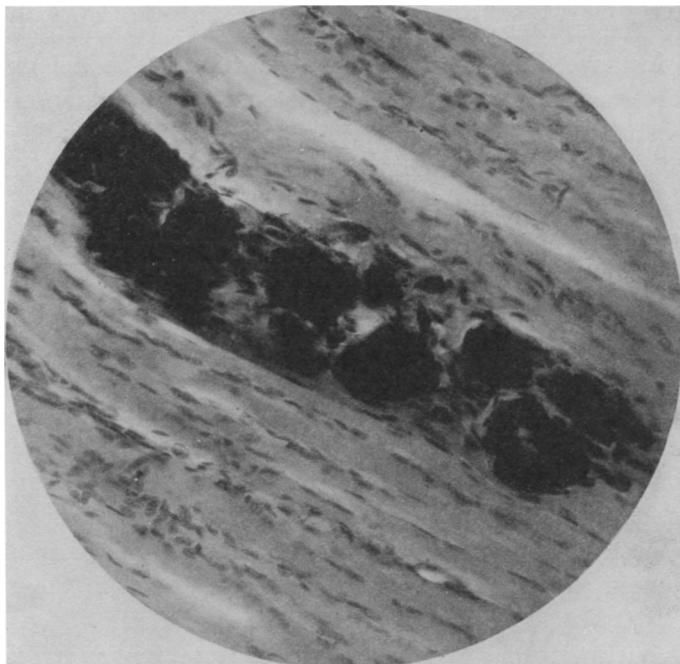


FIG. 4.—Microphotograph of lesion in muscle of dog. Death two weeks after injection. Healing stage. Note the almost complete absence of leukocytes. Hematoxylin and eosin.  $\times 230$ .

staining material in which bacteria are no longer demonstrable (Fig. 4).

Thirty-five rabbits were injected with strains from rheumatism. Seven of the 10 which died within 24 hours showed subendothelial hemorrhages of heart valves or papillary muscles. The valvular hemorrhages were smaller than those following injections of *Str. viridans*. They occurred most often in the following order: tricuspid, mitral and base of pulmonary and aortic cusps. Four showed myocardial hemorrhages, five pericardial hemorrhages, and six periarticular hemorrhages. Similar hemorrhages were observed in the rabbits which died soon after a second or third

injection. Endocarditis was observed in 20 out of the remaining 25 rabbits. The tricuspid valve alone was involved 11 times, the mitral alone four times, the aortic alone twice, both tricuspid and mitral endocarditis twice, and mitral and aortic endocarditis once. The vegetations were nearly always small subendothelial, grayish nodules which in the early stages were quite soft, smears now showing diplococci and leukocytes, and cultures, relatively large numbers of organisms. Later they became glistening white and quite firm, smears now no longer showing leukocytes nor bacteria. Healed endocarditis of the tricuspid valve was observed twice.

After animal passage or on otherwise modifying the strains large vegetations have been obtained which resemble those following injections of *Str. viridans*. The mode of origin of the experimental endocarditis is an embolic process just as in endocarditis due to *Str. viridans*. Myocarditis which was easily recognizable was observed eight times and almost without exception after the injection of strains which produced the lesions in the skeletal muscles. Acute pericarditis has been observed 11 times. Localized adhesive pericarditis twice. Pus was never found. The number of organisms in the exudate was always small. Adhesive pleuritis has been found once. In the 25 animals which lived long enough, arthritis was found 18 times. This was usually multiple, never suppurative, and the number of organisms was never very large. The tendency to healing was marked. The number of organisms was larger in the joints when the animals were injected with strains which produced muscle lesions. Endocarditis, pericarditis, myocarditis, and arthritis were observed six times in the same animal; endocarditis, pericarditis, and arthritis five times; endocarditis and arthritis six times; acute hemorrhagic or ulcerative appendicitis was observed five times. Suppurative appendicitis has not been observed. Acute hemorrhagic mesenteric lymphadenitis is very common following intravenous injections of these organisms. Scleral hemorrhages chiefly in the limbus occurred 10 times, in the retina twice and in the iris three times. Iritis, due to the organisms injected, has been observed twice. The ulcers of the stomach and the renal lesions will be described in separate papers. It should be said here that the strains from articular rheumatism which did not produce muscular lesions rarely produced a recognizable infection in the kidney, altho punctate hemorrhages in the cortex were observed in the animals which died in 24 to 48 hours after injection. The results following injections of the strains which produced muscle lesions were strikingly different. They produced hemorrhages in the medullary portion and pelvis of the kidney in the animals which died early, and almost constantly the picture of an "ascending nephritis" in both dogs and rabbits which died later.

Five rabbits (and two rats) all of which were injected either with the strains from rheumatism after one animal passage or with one strain of a streptococcus which had similar properties, showed symptoms of meningeal irritation during life. Two of the rabbits which died within 36 hours after injection showed small circumscribed meningeal hemorrhages. The other three rabbits and one rat which died spontaneously or which were chloroformed, five to 20 days after injection, showed localized grayish white nodular areas in the pia mater. Cultures from the crushed areas yielded the organism in pure culture in two of the rabbits. Cultures from the blood proved negative. Gross lesions in the brain substance have not been found but sections showed subcortical areas of round cell infiltration in two. The spleen in the animals injected with the strains from rheumatism as isolated has never been enlarged.

## EFFECT OF COLD ON RHEUMATISM.

It is a well-known fact that exposure to cold aggravates the symptoms in rheumatism. The results of the work of Poynton and Paine, of Beattie, and of myself show that this is true also in experimental rheumatism. As far as can be determined, however, no explanation has been advanced of this action of cold. Benedict and Slack<sup>1</sup> in a study on the fluctuations of temperature in different parts of the human body show that variations of from one to two or three degrees Centigrade are common. It occurred to me that the aggravation of symptoms in rheumatism might be due in part to the ability of these organisms to grow well at a low temperature as shown by the following experiments:

Test tubes 20 cm. in length with a caliber of 8 mm. were used. Long slants of ascites-dextrose agar covering the whole length of the tube were made and inoculated on the surface. The lower end was placed in running cold water and the upper end into a thermostat at 48° C. A control tube containing a thermometer to determine the temperature at the different levels was also included. The growth of six strains of pneumococci, three of hemolytic streptococci obtained from various sources, and seven strains of streptococci from rheumatism was studied. The average limit of growth of the pneumococci was 12 cm. from the bottom, at a temperature of approximately 32° C., of the hemolytic streptococci 8 cm., at a temperature of approximately 27° C., and of the strains of streptococci from rheumatism 6.5 cm., at a temperature of 22° C. The average point of optimum growth of the pneumococci was 18 cm. from the bottom, at a temperature of 38° C., of the hemolytic streptococci 16 cm., at a temperature of 37° C., and of the streptococci from rheumatism 12.5 cm., at a temperature of 35° C. Blood agar slants gave similar results. The ability, therefore, of the strains from rheumatism to grow at a low temperature is striking.

Frogs were inoculated with comparable doses of virulent pneumococci, hemolytic streptococci, and streptococci from rheumatism. Three frogs were inoculated intraperitoneally with each strain and three sets of experiments in which the frogs were kept at from 22° C. to 25° C. were made. Smears and blood agar plate cultures were made at intervals after inoculation. The results in the three sets of experiments were similar. The frogs receiving virulent pneumococci remained well and the organisms disappeared from the peritoneal cavity and blood. Three of the nine frogs injected with hemolytic streptococci died in two, three, and six days respectively. All of the frogs injected with streptococci from rheumatism died in from two to seven days from streptococcemia. The order of "virulence" of these strains here is exactly the reverse of that in warm-blooded animals. Knowing that the frog's temperature is that of its surroundings, similar experiments were made (except that approximately one-twentieth of the dose was given) in which the frogs were kept at 37° C. They were supplied with water and the incubator was well ventilated. Here the order of virulence was the same as for warm-blooded animals, the pneumococci killing most promptly, then

<sup>1</sup> Publication No. 155, Carnegie Institution, 1911.

the hemolytic streptococci, while those injected with streptococcus from rheumatism survived entirely or died latest. The control frogs lived indefinitely under the same conditions.

In connection with these experiments the following observation was made: The amount of phagocytosis in the smears from the blood and peritoneal exudate of the frogs injected with virulent pneumococci was strikingly greater in the experiments at a lower temperature than in the ones at 37° C., whereas in the case of the rheumatic streptococcus the reverse was true. This fact may be an additional reason why exposure to cold aggravates symptoms in rheumatism and suggests that the resistance or nonresistance to phagocytosis of bacteria may depend to a certain degree on the activity of growth—"growth pressure."

A board, in which two slits were sawed out at one end, was well padded and oiled with paraffin oil. A medium-sized rabbit (No. 457) was injected in the ear vein with the growth from 15 c.c. of rheumatism, Strain 735. It was now fastened to the padded board with a towel in such a way that the hind legs extended through the slits in the board. The right was placed in running water, the left kept at room temperature. The animal died the next day. The muscles and structures around the joints of the right leg showed numerous hemorrhages. No hemorrhages could be found above the line of exposure to cold and only a few in the opposite leg. The right knee and ankle joints gave a large number of leukocytes and many colonies while those from the left gave only a few. The blood contained a few bacteria.

The reasons then why exposure to cold aggravates the symptoms in rheumatism may be that the exposure tends to lower the temperature of the part directly and to cause vasoconstriction; the lowered temperature would favor the growth of the organisms directly, probably diminishing the activity of the leukocytes, and possibly increasing resistance to phagocytosis because of more active growth.

The results of cultures from the joints show that the organisms as found in man are very sensitive to oxygen pressure. Hence vasoconstriction besides tending to lower the temperature might favor the growth of the organisms by causing a lack of blood and leukocytes and in consequence a lowered oxygen pressure. Judging from experiments which I have made on the production of toxic substances during autolysis of pneumococci under various degrees of oxygen tension and from the results of Amberg and

Knox,<sup>1</sup> lack of oxygen in addition to favoring the growth of the organisms in rheumatism might increase markedly the amount of toxic substances produced during their disintegration; at the same time the ischemia from vasomotor constriction would tend to increase the concentration of the toxic substances.

#### CONCLUSIONS.

Three types of organisms have been isolated from the joints in rheumatism, each of which can be converted into the other quite readily.

Two types, one a very long chain producer, the other resembling a micrococcus, have been obtained from cases in which no muscle involvement was present. When injected into animals as isolated they commonly produce arthritis, endocarditis, and pericarditis, but not usually a visible myocarditis, and never a myositis.

The third type, a diplococcus with short chains of diplococci, was obtained from cases of rheumatism in which definite muscular involvement was present. It also produces arthritis, endocarditis, and pericarditis, but especially, marked myocarditis and myositis.

All three forms which have a low grade of virulence are quite freely susceptible to phagocytosis.

The results of the cultures in man and of the animal experiments support the view that acute articular rheumatism is due to streptococci which have peculiar properties.

Experimental and other evidence has been produced to indicate that the more or less closely related condition, muscular rheumatism or "rheumatic myositis," is due to streptococci, closely related to those in articular rheumatism.

The name *Str. rheumaticus* may be retained at present, not with the idea that the organisms so called always produce rheumatism, but rather to call attention to the fact that when streptococci produce the symptoms and lesions of rheumatism they have certain special features which streptococci from other sources do not usually have.

The affinity for joints, endocardium, pericardium, and often also myocardium and muscles which characterizes these organisms

<sup>1</sup> *Jour. Pharm. and Exp. Therap.*, 1912, 31, p. 223.

when first isolated, tends to disappear on cultivation. It may be restored by animal passage, and other strains of streptococci under certain conditions may be made to acquire the features of the strains from rheumatism. When the rheumatic strains have acquired the cultural features of hemolytic streptococci they lose the affinity for the endocardium and pericardium and acquire an even greater affinity for the joints. When they have been converted into pneumococci of a certain grade of virulence pulmonary hemorrhages and pneumonia are commonly found after intravenous injections whereas when the virulence is still greater death from pneumococcemia results. These and other facts suggest strongly the possibility that previous to an attack of rheumatism various types of the streptococcus group, especially hemolytic streptococci, acquire in the tissues of the infected individual the features which give them the simultaneous affinity for joints, endocardium, pericardium, and myocardium.

The experiments on mutation show that when these and other streptococci are grown in symbiosis with other bacteria, and under a low oxygen pressure they may acquire new features, and that sometimes they undergo marked changes on passage through animals. The places in the human body where such conditions prevail and where special features are likely to be acquired are parts of infection such as in the tonsils, various sinuses, the appendix, and about the gums and teeth. That this actually occurs in the tonsils in rheumatism seems quite clear; the mild character of the tonsillitis at the time of the attack and the late appearance of rheumatism in some cases of acute follicular (streptococcal) tonsillitis accord with this idea. The importance of focal infections as a point of entrance of bacteria in general is quite well recognized, but the idea that the focus serves in addition as a place where bacteria can acquire new properties is not generally recognized and needs to be emphasized.

Experimental evidence has been produced to show that probably lodgment of organisms in the fine capillaries of the iris occurs in rheumatic iritis. The lesions in the appendix, the diarrhea due to colitis, and the enlargement of the mesenteric lymph glands observed commonly in animals after intravenous injections of these

strains, as well as their isolation from the stool during rheumatism in man, indicate that the organisms may gain entrance through the lymph structures of the intestinal tract.

The strains from muscular rheumatism, especially after one or two animal passages, as well as other streptococci when they have attained a similar grade of virulence, show a marked affinity for the mucous membrane of the stomach, the pelvic mucous membrane and medullary portion of the kidney and the gall bladder. Ulcer of the stomach, the picture of an "ascending" nephritis, cholecystitis with beginning formation of gall stones, caused by streptococci, have been found repeatedly in rabbits and dogs injected with these strains, especially after one or more animal passages.